

Kuraishia molischiana* sp. nov., the teleomorph of *Candida molischiana

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Abstract

Thirty-two strains, many of them isolated from wood-associated habitats, and designated as *Kuraishia* (*Pichia*) *capsulata* and *Candida molischiana* according to their phenotype, exhibited two types of *Hae*III restriction fragment patterns of their small subunit rDNA with the neighboring ITS. One fragment pattern corresponded to that of the type strain of *K. capsulata*, whereas the other pattern was unique to the type strain of *C. molischiana*. Sequencing of the D1/D2 domain of the large subunit rDNA confirmed that the different *Hae*III restriction fragment patterns of small subunit rDNA with the neighboring ITS reliably distinguished *K. capsulata* from *C. molischiana*. Ascospore formation was observed in several *C. molischiana* strains and *K. molischiana* (type strain: NCAIM Y.01725, CBS 9993) is proposed as the teleomorphic state of *Candida molischiana*.

Introduction

Pichia capsulata (Wickerham) Kurtzman (1984a) was described as *Hansenula capsulata* by Wickerham (1951) based on strains isolated from frass in the tunnels of larvae underneath the bark of certain conifers in North America. Together with the other hat-spored *Hansenula* species, *H. capsulata* was transferred to the genus *Pichia* by Kurtzman (1984a). Based on the investigation of partial sequences of 18S and 26S ribosomal RNAs of the hat-spored, nitrate-assimilating *Pichia* species, Yamada et al. (1994) proposed the genus *Kuraishia* to accommodate *P. capsulata*. Currently, *Kuraishia capsulata* is the single species of the genus.

Candida molischiana (Zikes) Torula S.A. Meyer & Yarrow (Yarrow and Meyer 1978) was

described by Zikes (1911) as *molischiana*. The type strain of the species was isolated from used tanning bark. In the second edition of 'The Yeasts, A Taxonomic Study', *C. molischiana* was considered to be the anamorphic state of *P. capsulata* (Wickerham 1970). Since then, it has been considered so on the basis of the phenotypic similarity of the two taxa (Kurtzman 1984b, 1998). However, Lee and Komagata (1983) noted that the electrophoretic patterns of the cellular enzymes of the two species were different and raised the possibility that they may not represent an anamorph–teleomorph pair. This assumption was confirmed by Kurtzman and Robnett (1998), who found that sequences of the D1/D2 domain of large subunit (26S) rDNA of the type strains of the two species were different, thus demonstrating that *C. molischiana* is a distinct species.

In the present study, ascospore formation was observed in several *C. molischiana* strains. We propose placement of this new ascosporic species in the genus *Kuraishia* Yamada, Maeda & Mikata (Yamada et al. 1994), as *Kuraishia molischiana*, the teleomorph of *C. molischiana*.

Materials and methods

Organisms and physiological tests

The 32 strains investigated in this study are shown in Table 1. Phenotypic characterization of the strains was carried out according to the methods described by Yarrow (1998).

DNA restriction patterns and sequence analysis

*Hae*III restriction enzyme analysis of the small subunit rDNA with the neighboring ITS region was carried out according to Dlačny et al. (1999). The D1/D2 domain of the large subunit rDNA from selected strains was sequenced as described by Kurtzman and Robnett (1998), and those sequences were deposited with GenBank. A dataset of D1/D2 sequences from species closely related to *C. molischiana* was constructed by searching GenBank using the BLAST 2.2.10 database search program (Altschul et al. 1997) followed by sequence alignment using the ClustalX 1.81 program (Thompson et al. 1997). A phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei 1987) with *Schizosaccharomyces pombe* as the outgroup species. Bootstrap support for the tree was determined from 1000 replications.

Results and discussion

All *K. capsulata* and *C. molischiana* strains investigated during this study (Table 1) showed a very similar phenotype including colony appearance, microscopic morphology and assimilation spectrum. However, the *Hae*III restriction fragment patterns of the DNA fragments amplified with NS1 and ITS2 primers (from small subunit rDNA with the neighboring ITS region) from the strains under study formed two different groups (Figure 1). One restriction fragment pattern was characterized by an extra band compared to the

other type. The group of strains exhibiting the first pattern type included the type strain of *K. capsulata*, while the other group comprising strains with the extra band included the type strain of *C. molischiana*. To decide if the two types of restriction fragment patterns corresponded to *K. capsulata* and *C. molischiana*, the D1/D2 domain of the large subunit rDNA of 10 additional strains was sequenced (GenBank accession numbers are given in Table 1). NRRL Y-1889 and NRRL YB-2441 (*C. molischiana*) and NRRL YB-2520 (*K. capsulata*) exhibited 100% sequence identity in the D1/D2 region with the corresponding type strains and, for this reason, the sequences were not deposited in GenBank. The results of sequencing confirmed that the *Hae*III restriction fragment patterns of small subunit rDNA with the neighboring ITS region reliably distinguish *K. capsulata* from *C. molischiana*. The *Hae*III restriction fragment patterns of small subunit rDNA without the neighboring ITS region (amplified with NS1 and NS8 primers) made no distinction between the type strains of the two species indicating that the differentiating restriction site of the *Hae* III enzyme is situated on the ITS region.

The phylogenetic tree derived from neighbor-joining analysis of the D1/D2 domains of large subunit rDNA of the type strains of *K. capsulata*, *C. molischiana*, the teleomorph of *C. molischiana* and some related species, is shown in Figure 2. Two of the *C. molischiana* strains (NCAIM Y.01428 and NCAIM Y.01585) and one *K. capsulata* strain (NCAIM Y.01428) showed 1 or 2 substitutions, respectively, in their D1/D2 sequences compared to that of the type strains of *C. molischiana* and *K. capsulata*. The strain CBS 4327 exhibited three substitutions and one insertion compared to the type strain of *C. molischiana*, which renders them likely conspecific. However as Lee and Komagata (1983) detected more than 4% difference between the GC-content of the two strains, CBS 4327 is designated here as *Kuraishia cf. molischiana*. Further study is needed for clarifying the taxonomic status of this strain.

The proposal of the genus *Kuraishia* (Yamada et al. 1994), which was based on the comparison of partial rRNA sequences, was not initially accepted (Kurtzman, 1998) because the analysis included relatively few species, leaving uncertain whether this lineage was unique or part of a yet to be resolved larger clade. The study of Kurtzman and

Table 1. List of strains used in this study.

Species	Strain accession no.			GenBank accession no. D1/D2 large subunit	Source of isolation	Ascospores
	CBS ^a	NRRL ^b	NCAIM ^c			
<i>Kuraishia capsulata</i>	1993 ^T	Y-1842	Y.01230	U75516 ^(d)	Insect frass, conifer, Canada	+
	4306				Soil, Finland	+
		YB-2236			Lichen, Wyoming, USA	+
		YB-2512			Insect frass, black spruce, Ontario, Canada	+
		YB-2520			Insect frass, yellow spruce, Ontario, Canada	+
		YB-2754			Insect frass, fir, Tokyo, Japan	+
		YB-2980			Insect frass, larch, Germany	+
		YB-2988			Insect frass, larch, Germany	+
		YB-3002			Insect frass, fir, Germany	+
		YB-4665			Resin, fir, Quebec, Canada	+
	9987		Y.01458	AY937231	Rotten wood, black pine, Pilis Mountain, Hungary	—
	9988		Y.01571		Rotten wood, Scotch pine, Pilis Mountain, Hungary	—
	9989		Y.01726		Rotten wood, Norway spruce, Bükk Mountain, Hungary	+
<i>Kuraishia molischiana</i> (Including the anamorph: <i>Candida molischiana</i>)	136 ^{T(e)}	Y-2237		U70178 ^(d)	Used tanning bark	—
	836	Y-2238			Non-slimy variant of CBS 136	—
	837	Y-2234			Water, at 40 °C in wood-working factory, Sweden	—
	4683*		Y.01723*	AY937234	Soil	+
	5186				Non-mucoid variant of CBS 837	—
	7030				Soil, Japan	—
		Y-1889			Insect frass, red pine, Canada	+
		YB-2096			Insect frass, loblolly pine, Georgia, USA	+
		YB-2101			Insect frass, juniper, Montana, USA	+
		YB-2342			Insect frass, ponderosa pine, Washington, USA	+
		YB-2441			Insect frass, ponderosa pine, Washington, USA	+
		YB-2962			Insect frass, pine, Germany	+
		YB-3058			Insect frass, white pine, Wisconsin, USA	+
	9990		Y.01428*	AY937232	Fruiting body, mushroom (<i>Suillus</i> sp.) Pilis Mountain, Hungary	+
	9991		Y.01585	AY937233	Rotten wood, black pine, Pilis Mountain, Hungary	+
	9992		Y.01724		Rotten wood, black locust, Pilis Mountain, Hungary	+
	9993		Y.01725 ^{T(f)}	AY937235	Rotten wood, European beech, Pilis Mountain, Hungary	+
			Y.01736	AY937236	Faeces, domesticated rabbit, Budapest, Hungary	—
					Budapest, Hungary	—
<i>Kuraishia cf. molischiana</i>	4327		Y.01719	DQ026030	Soil, Finland	—

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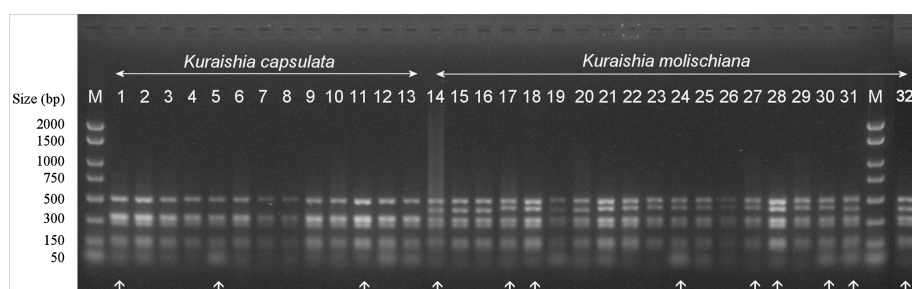


Figure 1. *Hae*III restriction analysis of small subunit rDNA with the neighboring ITS region. M: molecular size marker (Sigma P-9577), lane 1–13: *Kuraishia capsulata*; lane 1: CBS 1993^T, lane 2: CBS 4306, lane 3: NRRL YB-2236, lane 4: NRRL YB-2512, lane 5: NRRL YB-2520, lane 6: NRRL YB-2754, lane 7: NRRL YB-2980, lane 8: NRRL YB-2988, lane 9: NRRL YB-3002, lane 10: NRRL YB-4665, lane 11: NCAIM Y.01458, lane 12: NCAIM Y.01571, lane 13: NCAIM Y.01726; lane 14–16: *Kuraishia molischiana*; lane 14: CBS 136 (type strain of *C. molischiana*) lane 15: CBS 836, lane 16: CBS 837; lane 17: *Kuraishia cf. molischiana*, CBS 4327; lane 18–32: *Kuraishia molischiana*; lane 18: CBS 4683, lane 19: CBS 5186, lane 20: CBS 7030, lane 21: NRRL YB-2096, lane 22: NRRL YB-2101, lane 23: NRRL YB-2342, lane 24: NRRL YB-2441, lane 25: NRRL YB-2962, lane 26: NRRL YB-3058, lane 27: NCAIM Y.01428, lane 28: NCAIM Y.01585, lane 29: NCAIM Y.01724, lane 30: NCAIM Y.01725 (type strain of *K. molischiana*), lane 31: NCAIM Y.01736, lane 32: NRRL Y-1889 (the restriction pattern of this strain was obtained from a separate run). Those strains, whose D1/D2 domain sequences were determined are indicated with arrows.

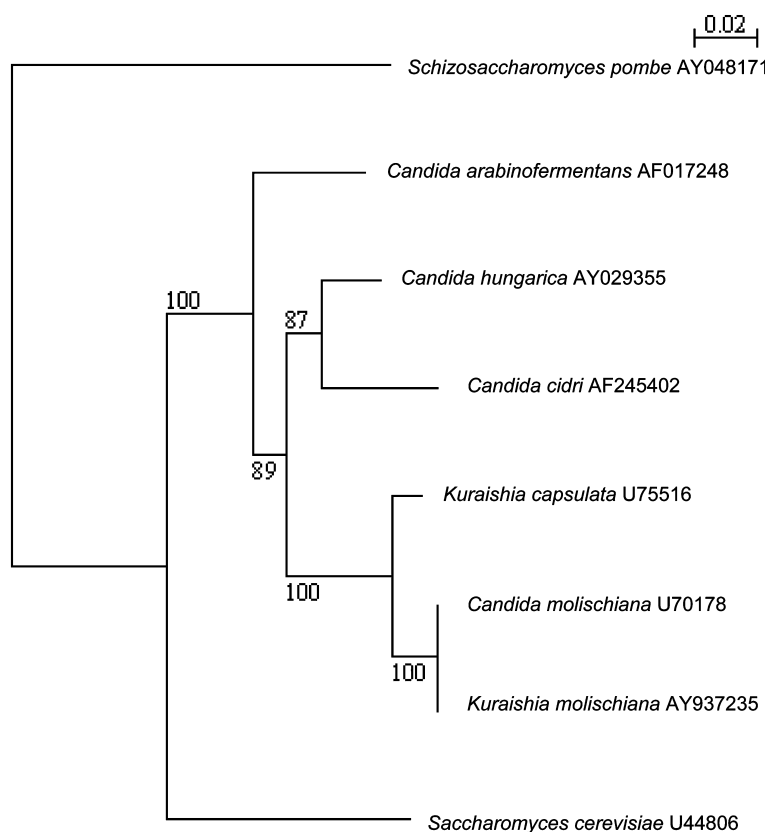


Figure 2. Phylogenetic tree showing the placement of *Kuraishia molischiana* and some closely related species based on analysis of the D1/D2 domain of large subunit rDNA. Sequences not generated during this study were obtained from GenBank. The tree was constructed by neighbor-joining analysis of aligned sequences. The numbers at nodes indicate the bootstrap values from 1000 replications. The scale bar shows the proportional sequence divergence.

Robnett (1998), in which all known ascomycetous yeasts were compared from divergence in the ca. 600 nucleotide sequence of domains 1 and 2 of large subunit (26S) rDNA, showed *K. capsulata* and *C. molischiana* to be members of a lineage that was isolated from other known ascospore genera, thus lending support to the proposal that the genus *Kuraishia* is unique. Furthermore, the recently described anamorphic species *Candida hungarica* and *C. cidri* have expanded the *Kuraishia* clade (Figure 2).

Kuraishia (Pichia) capsulata and *C. molischiana* were believed earlier to be conspecific because of their similar appearance in culture and their indistinguishable assimilation profiles (Kurtzman 1998). In this study, we were unable to find any clear-cut differences between the two taxa on standard physiological tests. Notably, most *C. molischiana* strains grew well at 37 °C, although two of them (NRRL YB-2342 and NRRL YB-2441) failed to do so. In contrast, most *K. capsulata* strains failed to grow at 37 °C, but one strain (NRRL YB-3002) grew weakly at this temperature.

Not all strains identified earlier as *K. capsulata* formed ascospores, but some strains initially classified as *C. molischiana* did form ascospores (Table 1). For some *C. molischiana* strains, in addition to the one or two-spored asci that are characteristic for *K. capsulata*, four-spored asci were also observed, although at rather low frequency.

Many of the strains examined share similar isolation sources, i.e., they were isolated from wood-associated habitats (Table 1). Wickerham (1951) isolated numerous *Hansenula capsulata* (*K. capsulata* and/or *C. molischiana*) strains from frass or tunnels of larvae underneath the bark of certain conifers, and it is likely that one of the primary habitats of the two species is the larvae of wood-boring insects.

Kuraishia molischiana Dlauchy, Péter,
Tornai-Lehoczki and Kurtzman sp. nov.

Status ascigerus *Candida molischiana* (Zikes) S.A. Meyer and Yarrow. In agar malti post dies tres in 25 °C cultura est mucoidea vel butyrosa, alba, glabra et nitida. Margine coloniae integro vel leviter undulato. Cellulae sunt sphaeroidae vel ellipsoideae (1.2–5.0 × 1.9–6.0 µm), singulae, binae vel raro racemis parvis conexas, undique gemmantes. In extracto malti post dies tres in 25 °C sedimentum formatur, pellicula non formatur. In agar

Zea mays confecto post dies 10 in 25 °C nec pseudohyphae nec hyphae formantur. Asci conjugati vel inconjugati et deliquescentes, 1–4 ascoporas piliformes, liberi habent.

D-glucosum et trehalosum fermentantur, D-galactosum, maltosum, sucrosum, lactosum, et raffinose, non fermentantur. D-glucosum, D-glucosaminum, N-acetyl-D-glucosaminum, D-ribosum, D-xylosum, L-arabinosum, D-arabinosum (variabile), L-rhamnosum (variabile), maltosum, trehalosum, α-methyl-D-glucosidum (lente, variabile), cellobiosum, salicinum, arbutinum, lactosum (tarde, exigue, variabile), melezitolum (variabile), amyllum solubile, glycerolum, meso-erythritolum, ribitolum, xylitolum, L-arabinitolum, D-glucitolum, D-mannitolum, 2-ketogluconicum, D-gluconicum (variabile), succinatum, (variabiles) methanolum (variabiles), ethanolum propane-1, 2-diolum (variabile) assimilantur, at non D-galactosum, L-sorbose, sucrosum, melibiosum, raffinose, inulinum, galactitolum, myo-inositolum, D-glucuronicum, D-galacturonicum, DL-lactatum, citratum, saccharatum, butane-2,3-diolum, hexadecanum. Kalium nitricum, natrium nitrosum, ethylaminum hydrochloricum, lysinum, cadaverinum dihydrochloricum, glucosaminum assimilantur at non creatinum, creatininum, imidazolium.

Materia amyloidea iodophila non formatur. Vitamina externa crescentiae sunt necessaria. Crescere potest in 30 °C, in 37 °C (variabile), at non in 45 °C. In agar extracto fermenti confecto 50 partes glucosi per centum non crescit. Parte 0,1 cycloheximidi per mille crescit (variabile). Ureum non finditur. Diazonium caeruleum B est negativum.

Typus strips NCAIM Y.01725 (CBS 9993).

Description of Kuraishia molischiana Dlauchy,
Péter, Tornai-Lehoczki and Kurtzman sp. nov.

Growth on 5% malt extract agar. After 3 days at 25 °C, the streak culture is mucoid or butyrous, tannish-white, smooth and glistening. The margin is entire or slightly undulating. Cells are spherical to ellipsoidal, 1.2–5.0 × 1.9–6.0 µm. Vegetative reproduction proceeds by multilateral budding. Cells are single, in pairs and, rarely, in small clusters.

Growth in 5% malt extract. After 3 days at 25 °C, coherent sediment and usually a thin ring are present, the latter becomes more pronounced after prolonged incubation. Pellicles are absent.

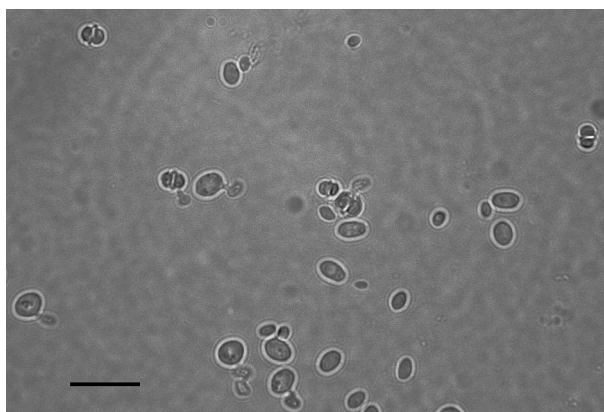


Figure 3. *Kuraishia molischiana* NCAIM Y.01725. Ascosporeulating culture on cornmeal agar, 5 days, 25 °C. Bar = 10 µm.

Dalmau plate on cornmeal agar. After 10 days at 25 °C, neither pseudohyphae nor septate hyphae are formed.

Formation of ascospores. In sporulating strains, conjugation of independent cells or parent cell–bud conjugation usually precedes spore formation, but in some strains unconjugated asci also occasionally occur. One to four easily liberating hat-shaped ascospores are formed per ascus. Two-spored asci are predominant (Figure 3), however, four-spored asci were also observed, though very rarely and only in three strains. The presence of heterogamous conjugation is suggestive of homothallism. Ascospores were observed after 5–10 days at 25 °C, on at least one of the following media: YM, 5% malt extract and cornmeal agars.

Physiological characteristics. The results of the physiological characteristics tested are shown in Table 2.

Type. The type strain was recovered from rotten wood of *Fagus sylvatica* in Pilis Mountain (near Budapest) in Hungary and is maintained as NCAIM Y.01725 (CBS 9993) in the National Collection of Agricultural and Industrial Microorganisms in Budapest (Hungary). The origin of the other strains is shown in Table 1.

Etymology. The species epithet *molischiana* was selected to emphasize that this species is the teleomorph pair of *Candida molischiana*.

Acknowledgements

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Table 2. Characteristics of *Kuraishia molischiana*.

<i>Fermentation</i>	
D-Glucose	+ or s
D-Galactose	–
Sucrose	–
Maltose	–
Lactose	–
Raffinose	–
α,α-Trehalose	+ or s
<i>Utilization of carbon sources</i>	
D-Glucose	+
D-Galactose	–
L-Sorbose	–
D-Glucosamine	+
N-Acetyl-D-glucosamine	+ or s
D-Ribose	+
D-Xylose	+
L-Arabinose	+ or s or l
D-Arabinose	v
L-Rhamnose	v
Sucrose	–
Maltose	+
α,α-Trehalose	+
Methyl-α-D-Glucoside	s or –
Cellobiose	+
Salicin	+
Arbutin	+
Melibiose	–
Lactose	– or l,w
Raffinose	–
Melezitose	v
Inulin	–
Starch	+
Glycerol	+
meso-Erythritol	+
Ribitol	+
Xylitol	+
L-Arabinitol	+
D-Glucitol	+

Table 2. Continued.

<i>Fermentation</i>	
D-Mannitol	+
Galactitol	—
myo-Inositol	—
2-keto-D-Gluconate	+
D-Gluconate	v
D-Glucuronate	—
D-Galacturonate	—
DL-lactate	—
Succinate	v
Citrate	—
Saccharate	—
Methanol	v
Ethanol	+
Propane 1,2 diol	v
Butane 2,3 diol	—
Hexadecane	—
<i>Utilization of nitrogen sources</i>	
Potassium Nitrate	+
Sodium Nitrite	+
Ethylamine hydrochloride	+
L-Lysine	+
Cadaverine dihydrochloride	+
Creatine	—
Creatinine	—
Glucosamine	+
Imidazole	—
Growth in vitamin-free medium	—
Growth at different temperatures:	
30 °C	+
35 °C	v
37 °C	v
40 °C	v
42 °C	v
45 °C	—
Growth on 50% w/w glucose yeast extract agar	—
Growth in 10% NaCl and 5% glucose in yeast nitrogen base	v
Growth in 16% NaCl and 5% glucose in yeast nitrogen base	—
Growth with 0.01% cycloheximide	v
Growth with 1% acetic acid	—
Formation of amyloid material	—
Hydrolysis of urea	—
Colour reaction with Diazonium Blue B	—

s = slow; l = latent; w = weak, v = variable.

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